Another type of aneuploidy is triploidy. A triploid individual has three of every chromosome, that is, three haploid sets of chromosomes. A triploid human would have 69 chromosomes (3 haploid sets of 23), and a triploid dog would have 117 chromosomes. Production of triploids seems to be relatively common and can occur by, for example, fertilization by two sperm. However, birth of a live triploid is extraordinarily rare and such individuals are quite abnormal. The rare triploid that survives for more than a few hours after birth is almost certainly a mosaic, having a large proportion of diploid cells.

A chromosome deletion occurs when the chromosome breaks and a piece is lost. This of course involves loss of genetic information and results in what could be considered "partial monosomy" for that chromosome.

A related abnormality is a chromosome inversion. In this case, a break or breaks occur and that fragment of chromosome is inverted and rejoined rather than being lost. Inversions are thus rearrangements that do not involve loss of genetic material and, unless the breakpoints disrupt an important gene, individuals carrying inversions have a normal phenotype.

In a monosomic sample, with 2n-1 chromosomes, one entire chromosome and all its loci are lost. Similarly, in a 2n+1 trisomic sample, one extra chromosome is present in each cell, meaning one specific chromosome is represented three times due to a non-disjunction event, usually in the female gametogenesis. A similar, but more pronounced, situation occurs in the case of a triploid sample in which each chromosome is represented three times instead of twice in each cell.

Pregnancies can be established in infertile women using the technique of *in-vitro* fertilization (IVF). In spite of the high rate of fertilization *in-vitro*, the rate of pregnancy following these procedures is relatively low, ranging from 15% to 25%. Cytogenetic studies of human oocytes fixed after failing to fertilize *in-vitro* display a relatively high incidence of chromosomal abnormalities (aneuploidy). Also, studies of many spontaneous abortions and pre-term embryos show that chromosomal abnormalities may be the main

cause of fetal loss. The frequency of chromosomal abnormality in embryos generated using IVF is much higher than total abnormalities reported for sperm and oocytes.

In the IVF procedure, aneuploidy is the most frequently observed abnormality in the embryos generated. Many reports strongly indicate that chromosomal aneuploidy is the prime cause of fertilization failure in oocytes and implantation failure of embryos. Aneuploidy mainly arises during meiotic non-disjunction; but many environmental factors may also disrupt spindle function and eventually lead to the formation of aneuploid embryos.

Using methods currently known in the art to assess the embryo's gross chromosome makeup, one would perform cytogenetic analyses, such as karyotyping. However, this method is not a practical solution for single cells, and therefore cannot be performed as a pre-implantation screen.

Therefore, there is a need to develop rapid, inexpensive, automatable methods for detecting aneuploidy in an embryo that can be applied in the pre-implantation setting for *in-vitro* fertilization. The present invention provides a method, which has application, *inter alia*, as a rapid, single-tube method for the simultaneous detection of aneuploidy in one, multiple or all chromosomes of a subject.

In particular, this method may increase the success rates of IVF, as embryos with aberrant chromosome numbers (aneuploid) could be screened out by a pre-implantation scan of the embryogenic genetic component.

PCT/AU2004/000894
Received 24 March 2005

of the sample and standard. Accordingly, this provides simultaneous measurements for the relative frequency of multiple chromosomes in a sample.

In one aspect, the number of polynucleotides bound to a microparticle derived from a specific chromosome may be from about 1 to about 40,000. In a preferred aspect, the number of polynucleotides bound to the microparticle is from about 1 to about 3,000. In a most preferred aspect, the number of polynucleotides bound to a microparticle is about 2,000.

The method of the present invention has application to the detection of aneuploidy in *any* organism.

In preferred embodiment of the present invention, the subject is a human or other animal embryo generated using *in-vitro* fertilization.

The method of the present invention is able to detect aneuploidy in DNA extracted and/or amplified from a single cell. Therefore, the method of the present application is suitable, *inter alia*, for the detection of aneuploidy in animal embryos generated using *in-vitro* fertilization, prior to implantation of said embryo.

In addition to the detection of chromosome number in an organism, the present invention has application for the detection of non-disjunction events in reproductive cells.

The present invention further provides a kit useful for simultaneously detecting aneuploidy for multiple chromosomes in organism, embryo or reproductive tissue.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for the detection and sorting of microparticles in a mixture of microparticles. The method of the present invention allows for the detection and sorting of many distinct microparticle classes. Detection and sorting is on the basis of microparticle size, the fluorescence spectrum of any attached reporter molecule, the fluorescence intensity of the reporter molecule and discrimination of events based on particle number. These microparticle classes have particular application as binding agents for the detection of aneuploidy in an organism or embryo of the organism. In humans, the detection and sorting of at least 24 classes of microparticles would be sufficient for a single tube method for the simultaneous detection of aneuploidy in all chromosomes, wherein each distinct microparticle class comprises a polynucleotide sequence complementary to, and specific for, a polynucleotide sequence that is unique to a particular human chromosome. Furthermore, using currently available technology, the present method has application for the simultaneous detection of aneuploidy in all chromosomes for an organism that has 216 or fewer pairs of chromosomes. Kits for the simultaneous detection of aneuploidy in one or more human chromosomes are also provided.

Before describing the present invention in detail, it is to be understood that unless otherwise indicated, the subject invention is not limited to specific formulations of agents, manufacturing methods, methodologies, or the like, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

As used in the subject specification, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "a microparticle" includes a single microparticle, as well as two or more microparticles.

In describing and claiming the present invention, the following terminology is used in accordance with the definitions set forth below.

Current methods in the art for the detection of aneuploidy in embryos include post-implantation screens. Jenderney *et al.* (*Mol. Hum. Reprod.* 6(9): 855-860, 2000) describe the method of using QF-PCR, specific for short tandem repeats on specific chromosomes, on samples of amniotic fluid. It is also possible to assess potential aneuploidy in a fetus from fetal cells in the maternal blood stream, using techniques such as fluorescent *in-situ* hybridization (FISH) (Bianchi *et al.*, *Prenat. Diag.* 22(7): 609-615, 2002). However, as can be seen from the material sampled in these studies, these techniques are only suitable for the detection of aneuploidy in an embryo or fetus post-implantation.

The method of the present invention is able to detect aneuploidy in DNA extracted and/or amplified from a single cell. Therefore, the method of the present application is suitable, *inter alia*, for the detection of aneuploidy in animal embryos generated using *in-vitro* fertilization, prior to implantation of said embryo.

Single cells may be isolated from embryos using standard blastomere biopsy techniques, as will be known to those of skill in the art. Briefly, the blastomere biopsy procedure comprises the following steps:

(i) A 7-cell embryo, on Day 3 after IVF, is ready to be biopsied. It is held in place on a micromanipulator with a holding pipette.

(ii) A zona drilling pipette is used to drill a hole through the shell of the embryo (the zona) using acid Tyrode's.

(iii) The embryo biopsy pipette is then introduced through this opening, and gentle suction is applied to dislodge a single cell (a blastomere) from the embryo.

(iv) The biopsied embryo is then returned to the incubator for further culture. The blastomere can now be screened for aneuploidy according to the method of the present invention.

(v) Based on the analysis of the blastomere, corresponding non-aneuploid embryos are then selected for implantation.

Accordingly, the present invention provides a method for the detection of aneuploidy in an animal embryo generated by *in-vitro* fertilization, prior to implantation of the embryo.

In a preferred embodiment of the present invention, the animal embryo is a human embryo.

In addition to the detection of chromosome number in an organism, the present invention has application for the detection of non-disjunction events in reproductive cells. In this aspect of the present invention, gametes of a said organism, preferably a human, may be tested for missing and/or duplicated chromosomes. The method of this aspect of the present invention would be largely similar to the methods hereinbefore described. Briefly, a nucleic acid representative of a given chromosome in a gamete is labelled with a reporter molecule such as a fluorescent marker, while an equivalent representative polynucleotide from a known non-aneuploid gamete is labelled with a different fluorescent marker. As with the method described for detection of aneuploidy in a somatic or embryogenic cell, the sample and standard polynucleotides are competitively bound to a limiting number of binding agents. A missing chromosome in the sample would be manifest as an increased detection of the standard on the binding agent. Duplication of a chromosome in the sample would be detected as an increased binding of sample to the binding agent. In the case where no non-disjunction events have occurred in the sample, binding of the standard and sample to the binding agent should be approximately equal.

Binding agents contemplated by the present invention comprise a polynucleotide sequence immobilised to a substrate. The polynucleotide sequence of the binding agent comprises a polynucleotide sequence that is complementary to the nucleic acid sequence of the sample and standard, as described *supra*.

By complementary, it is to be understood that an immobilized polynucleotide of the present invention should bind to a chromosome-number representative polynucleotide of